REMARKS

Claims 10-14, 16, 18, 19 and 22-27 are pending. Claims 18, 19 and 22-27 have been withdrawn. Claims 15, 17 and 28 have been cancelled without prejudice. Applicants reserve the right to file a divisional applications relating to the cancelled subject matter. Claims 10 and 16 have been amended. Specifically, claim 10 has been amended to define paramagnetic CEST agent comprising a substrate molecule (SH) endowed with at least one mobile proton in exchange with bulk water bound by means of electrostatic interactions to a paramagnetic chelate complex (SR). The support for the amendments may be found throughout the specification and specifically at pages 3 and 4 of the specification.. No new matter has been added.

35 U.S.C. § 101(a)

Claim 28 was rejected under 35 U.S.C. § 101(a). In view of cancellation of claim 28, this rejection is moot and should be withdrawn.

35 U.S.C. § 112

Claims 10-17 are rejected under 35 U.S.C. 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicants respectfully disagree.

Firstly, Applicants respectfully direct the Examiner's attention to claim 10. Specifically, instant specification defines "mobile proton" as a proton that may exchange with the surrounding protons, i.e., the proton of the bulk water (see claim 1 and page 1, line 8 of the specification as originally filed. Further, in response to the Examiner's comments on page 3 of the Office Action, Applicants urge that the substrate molecule (SH) is (the subject) bound to the paramagnetic chelate complex (SR).

Further, Applicants respectfully direct the Examiner's attention to claim 16 where the Examiner alleged a lack of units for the provided Ka value. In response, Applicants urge that from a thermodynamic point of view, the constant Ka value of claim 16 is dimensionless. In the instnat case, in fact, the Ka unit would depend on the stoichiometry of the reaction. By using the activity, a dimensionless value is obtained that can be used as a reference regardless of the reaction stoichiometry.

In view of the presented remarks, Applicants request reconsideration and withdrawal of the rejections.

Claim objections.

Claim 10 is objected to because of the informalities. In view of amendments to claim 10, this objection is moot and should be withdrawn.

35 U.S.C. § 103(a)

.The instant invention *inter alia*, relates to the technical field of the CEST based MRI. In particular it refers to adducts between magnetic resonance shift reagents (SR) and substrates containing exchangeable protons (SH) for CEST applications and an imaging method using them in CEST based MRI procedures (claim 1 and page 8).

Firstly, Applicants would like to emphasize that CEST-based MRI and conventional MRI are well different imaging techniques, that are based on totally different principles, and thus exploit different parameters and take advantage from different contrast agents to provide different information.

In fact, one skilled in the art recognizes that conventional MRI imaging is based on and exploits differences existing on the T1 and T2 relaxation times of the tissue protons (or, that is the same, on the proton relaxation rate R1 and R2 being R1 = 1/T1);

indeed, the relaxation times T1 and T2 are the crucial parameters of this technique. Further, the contrast recorded in conventional MRI is the result of differences in relaxation times T1 and T2 of tissue protons, mainly water protons, and depends on the concentration and on the relaxivity of the paramagnetic complex: the higher is the contrast agent relaxivity, the stronger is the contrast it provides.

Also, contrast agents for use in conventional MRI act by affecting, in particular by shortening, the relaxation times T1 or T2 of the surrounding water protons. Typically they include a paramagnetic metal ion, generally gadolinium(Gd3+) that acts by reducing the relaxation time, especially T1, of surrounding water protons. The higher is the operated T1 reduction, the stronger is the recorded signal and the brighter appear the enhanced region/tissue in the recorded T1 weighted image. Finally, conventional MRI makes use of a single radiofrequency (rf) field and provides contrasted images of organ or tissues. Notably, commercial contrast agents for use in conventional MRI imaging, including for instance, Gd-DTPA, Gd-DOTA, Gd-HPDO3A, Gd-EDTA, and Gd-BOPTA do not contain exchangeable protons.

In contrast, the MRI-CEST is a technique based on the irradiation of a mobile proton saturating it. Accordingly, the crucial parameter to consider in CEST imaging is the proton irradiation frequency, whose value is defined by the proton chemical shift, and the basic requirement of CEST contrast agents is the presence of mobile protons having a low mean exchange rate with water protons. Further, for the CEST imaging, a proper off-resonance radiofrequency is applied to saturate the mobile protons of the contrast agent.

The saturation is then transferred from the contrast agent's protons to bulk water protons through a cross relaxation process and/or chemical exchange, determining a neat reduction of the bulk water signal, which is registered in the CEST based MRI technique, the contrast that relies on differences in the water signal intensity caused by differences in the extent of the saturation transfer ST. Further, CEST imaging does not result on enhanced images of organs or tissues, but it normally provides measures of physical-chemical parameter of diagnostic interest. Finally, on the contrary to the conventional MRI, the CEST based MRI technique makes use of a second in the field that is responsible for the saturation of the exchangeable pool of protons that exchange with water. This is in contrast to the conventional MRI where there is no proton irradiation, nor saturation and not even transfer of the saturation.

Because of the above fundamental differences, it is clear to one person skilled in the art, that parameters, contrast agents and imaging conditions positively affecting conventional MRI imaging are not expected to provide any advantage or, even, any contrast in CEST based MRI imaging. Therefore, one skilled in the art would be perfectly aware that teaching and suggestions validly applicable to improving conventional MRI imaging are not equally applicable in CEST imaging and, therefore, there is no expectation of success in e transferring them to CEST MRI.

Claims 10-12 and 15-17 were rejected as obvious over the combination of Balaban et al., (WO 00/66180, "Balaban") in view of Aime et al., (Magnetic Resonance in Medicine, 2002, "Aime"). In view of the remarks presented below and amendments to claims 10, Applicants request reconsideration and withdrawal of the obviousness rejection.

In order to establish obviousness, it is necessary, *inter alia*, to (i) determine the scope of the prior art and (ii) the differences between the claimed subject matter and that of the prior art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Furthermore, a *prima facie* finding of obviousness cannot be established when the "improvement is more than the predictable use of prior art elements according to their established functions." *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct 1727, 1739 (2007). Also, the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP 2142 and 2143.03. A reasonable expectation of success is required. MPEP 2143.02.

The amended claim 10 is directed to a method of CEST imaging that comprises administering into a subject a paramagnetic CEST agent comprising a substrate molecule (SH) endowed with at least one mobile proton in exchange with bulk water bound by means of electrostatic interactions to a paramagnetic chelate complex (SR). The improvement provided by the method of the invention relies on the use of paramagnetic CEST agents comprising a paramagnetic chelate complex (SR) that is bound by means of electrostatic interactions to a substrate molecule (SH) endowed with mobile proton(s) in exchange with bulk water and that, as a result of the said non-covalent binding interaction, suitably shifts the chemical shift of the (SH) mobile proton(s) to be irradiated to observe saturation transfer (see page 2, lines 18-21 of the specification.). Because the said shift action exerted on the mobile proton by the non-covalently interacting paramagnetic complex, the separation between the resonance frequency of the mobile proton(s) belonging to the substrate (SH) and that of the exchanging bulk water protons is increased (see page 2, lines 11-12 of the specification),

thus avoiding protons coalescence and allowing for an enhanced efficiency of saturation transfer (see from page 1, line 24, to page 2, line 2 of the specification).

Balaban relates to an imaging method that comprises using CEST agents. The CEST agents disclosed by Balaban are diamagnetic compounds endowed with mobile protons (claim 7) or mixtures thereof (page 14, line15-20). Further, the use of "metals" to enhance the small shift of protons in biomolecules is mentioned (page 23, lines 25-28). Balaban fails to teach or suggest paramagnetic CEST agents, as well as an imaging method wherein a paramagnetic CEST agent is used with CEST based procedures. Further, Balaban fails to teach or suggest a paramagnetic CEST agent comprising a paramagnetic chelate complex bound by means of electrostatic interactions to a substrate molecule endowed with mobile proton(s) and an imaging method wherein a non covalent interaction between a paramagnetic complex and a substrate molecule is exploited to increase the separation between the resonance frequency of the mobile proton(s) to be irradiated and the bulk water. Thus, Balaban alone or in combination does not disclose, teach or suggest an instant invention.

Aime does not cure the deficiencies of Balaban. Aime relates to DOTAM-GLY ligand (wherein GLy stands for glyicinamide), their Ln(III) complexes and their use as pH-sensitive CEST agents. These paramagnetic complexes possess exchangeable protons that are provided by the metal coordinated water protons and by the amide protons of the ligand structure (see, for instance the abstract and mid-page 643, right column), that is an amide group covalently linked to the chelating molecule. Thus, Aime fails teach or suggest instant non-covalent adducts and to their use in the CEST based

imaging method. Applicants urge that Balaban in view of Aime does not teach or suggest an instant invention.

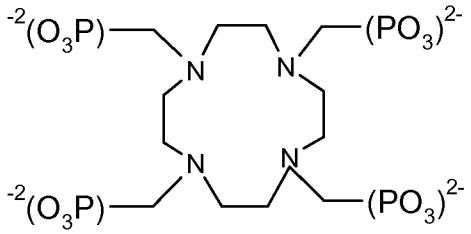
Claim 13 was rejected as obvious over the combination of Balaban et al., (WO 00/66180) in view of Aime et al., (Magnetic Resonance in Medicine, 2002) and in further view of Frullano et al., (Topics in Current Chemistry, 2002), "Frullano". The Examiner contends that "it would have been prima facie obvious to one of ordinary skill in the art to use the combination of $[LnDOTP]^{4-}$ taught by Frullano with polyarginine taught by Balaban in order to form CEST agents with higer relaxivity that either $[LnDOTP]^{4-}$ or polyarginine alone had yielding higher contrast in the CEST based MRI images." (see Office Action at page 9, bottom). Applicants respectfully disagree and in view of the remarks presented below and amendments to claims 10, request reconsideration and withdrawal of the obviousness rejection.

As discussed above, Balaban and Aime do not teach or suggest instant methods. Frullano does not remedy the deficiencies of Balaban and Aime. Frullano relates to relates to the technical field of the (conventional) MRI imaging. In particular, Frullano relates to configuration and conformations of Ln^{3+} complexes useful as contrast agents in MRI (see the Abstract and page 26, the Introduction chapter) and discusses paramagnetic $[LnDOTP]^{4-}$ among macrocyclic complexes.

Thus, Applicants respectfully point out that Frullano relates to a technical field and to diagnostic technique (conventional MRI) other than that (CEST based MRI) used in the method of the instant claim 13. To this extent, Applicants direct the Examiner's attention to the formerly discussed significant differences existing from conventional and

CEST based MRI imaging and to the consequent lack of any expectation of success in transferring any teaching or suggestion from one technique to the other.

Further, Frullano does not teach or suggest that "$[LnDOTP]^{4-}$ has functional groups with mobile protons". Indeed $[LnDOTP]^{4-}$ complexes include a chelating ligand of formula



that does not include any mobile proton and while they exhibit high relaxivity (mid-page 43) and may thus act as conventional MRI contrast agents, they may not be used as CEST agents in CEST based MRI procedures. Thus, one skilled in the art, familiar with the fact that the basic requirement of CEST contrast agents is the presence of mobile protons would have never conceived using them as a CEST agent in CEST imaging procedures.

Thus, Frullano fails to remedy the deficiencies of Balaban and Aime. Applicants request reconsideration and withdrawal of the obviousness rejection of claim 13.

Claim 14 was rejected as obvious over the combination of Balaban et al., (WO 00/66180) in view of Aime et al., (Magnetic Resonance in Medicine, 2002) and in further view of Frullano et al., (Topics in Current Chemistry, 2002) and in further view of Aime et al. (JACS, 1995) ("Aime I") In view of the remarks presented below and amendments

to claims 10, Applicants request reconsideration and withdrawal of the obviousness rejection.

As discussed above, Balaban, Aime and Frullano fail to teach or suggest instant methods. Aime I fails to cure the defects of Balaban, Aime and Frullano.

The instant claim 14 relates to the method of CEST imaging that comprises administering a paramagnetic CEST agent comprising a substrate molecule (SH) bound by means of electrostatic interactions to a paramagnetic chelate complex (SR) in which the substrate molecule (SH) and the paramagnetic chelate complex (SR) are compartmentalized in biocompatible systems selected from liposomes, nanoparticles, microemulsions and protein cavities.

The Examiner conceeds that Balaban, Aime and Frullano do not teach using the contrast agents in (that is, within) liposomes, microemulsion or protein cavities (see Office Action, page 10) but contends Aime I teaches covalently or non-covalently binding the macrocycle $[LnDOTP]^{4-}$ and other lanthanide complexes into macromolecular systems such as polylysine…..micelles and haemoglobin, and also disclose including lanthanide complexes into larger slower tumbling systems yields large relaxation enhancements for the lanthanide systems (see Office Action, bridging pages 10 and 11).

Applicants respectfully disagree. AimeI relates to convention MRI large relaxation enhancements "observed ……. by covalently linking Gd complexes to macromolecular systems (such as albumin, polylysin and dextran) and by inducement of noncovalent interactions between slowly tumbling substrates (such as micelles and albumin) and suitable functionalities on the surface of the ligand (see page 9365, left column). AimeI does not teach **inclusion** of macrocyclic $[LnDOTP]^{4-}$ into

macromolecular systems. Thus, AimeI does not remedy the deficiency of Balaban, Aime, and Frullano and does not teach or suggest instant methods.

In view of the remarks presented below and amendments to claims 10, Applicants request reconsideration and withdrawal of the obviousness rejection.

Obviousness Double Patenting Over USSN 10/502701

Claims 10-17 and 28 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being allegedly unpatentable over claims 1-4,6-8,10,13,14, and 18 of co-pending Application No. 10/502701. Applicants request putting this rejection on hold until the other application issues.

CONCLUSION

Having addressed all outstanding grounds for rejection, applicants respectfully maintain that presently pending claims are in condition for allowance. Applicants therefore request the speedy issuance of a notice of allowance.

If a telephone interview would be of assistance in the prosecution of this application, the Examiner is invited to telephone Applicants' undersigned attorneys at his convenience at the number provided below.

No fees are believed due in connection with the filing of this Amendment and Response. However, the Director is hereby authorized to charge any required fees and credit any overpayments to Deposit Account No. **50-0540**.

Respectfully submitted,

Date: June 21, 2010

/Ilona Gont/

Ilona Gont, Reg. No 58,714
Donald L. Rhoads, Reg. No. 34,705
Kramer Levin Naftalis & Frankel LLP
1177 Avenue of the Americas
New York, N.Y. 10036
(212) 715-9100 (telephone)
(212) 715-8000 (facsimile)